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CAPILLARY ELECTROPHORESIS: BIOTECHNOLOGY FOR SEPARATION
OF DNA AND CHROMOSOMES

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Introduction

Electrophoresis has been used for the separation of particles, ions and molecules for a number of years. The technology for separation and detection of the results has many applications in the life sciences. One of the major goals of the scientific community is to separate DNA molecules and intact chromosomes based upon their different lengths or number of base pairs. This may be achieved using some of the commercially available and widely used methods, but these processes require a considerable amount of time. The challenge is to achieve separation of intact chromosomes in a short time, preferably in a matter of minutes.

The Biophysics Branch of the Marshall Space Flight Center has been doing electrophoresis research for several years. Recently the research effort has focused on macromolecules, particularly DNA. Electrophoretic mobilities of various DNA molecules have been determined using new technology. The research team has worked with agarose and polyacrylamide gels; chambers made from microslides, glass, and plastics; various buffer systems; ac and dc fields; and advanced technology for imaging and videotaping of various sizes of DNA in Brownian motion and under the effects of an electric field. This has been accomplished by using epifluorescence, a CCD camera with an image intensifier, an image analysis system, and a research microscope (1).

The technology for capillary electrophoresis (CE) has developed rapidly and has many advantages over more conventional methods. Separation of DNA and chromosomes has required the use of gels. A large portion of the science of modern electrophoresis is devoted to understanding and controlling the formation of types of gels. However, the use of these gels has not been easily adapted to on-line sample applications, detection, quantification or automated operation. This type of electrophoresis has been for a number of years a powerful but manual-intensive methodology (2).

Capillary electrophoresis has been used to separate various types of biologicals including purine bases and nucleosides (3), drug related impurities (4), amino acids, human urine, peptides from the tryptic digestion of egg white lysozyme, and the components of a one kilobase ladder of DNA in agarose solutions (5). Entangled polymer solutions have been employed as a mesh for separating DNA restriction fragments ranging in size from 72 to 1353 base pairs (6). Grossman and Soan have used CE to study the orientation effects on the electrophoretic mobility of the tobacco mosaic virus in free solution (7). This brings us to a brief discussion of the advantages of capillary electrophoresis and, therefore, the technique of choice for this research. Some of these advantages are as follows: sample requirements of only 2 to 4 nL; time requirements of only 10 to 40 minutes; high resolution of sample species; reproducibility is greatly enhanced with only about 0.5 percent error; sensitivity in the picomoles range; no molecular weight limitations enabling separation of smaller substances from complex macromolecules; capillary conditions may be mild with the use of mobile phases usually ranging from slightly acidic to slightly basic buffers and certain organic solvents and denaturing agents may be added; compatibility with biological systems provides for recovery of more than 95 percent of original enzymes and their immunological activities; instrumentation for CE may be programmed for semi-automated or fully automated operation; complete spectrum may be measured after a single injection; and minimal reagent consumption is a decided advantage(8).

Experimental Section

The current task at MSFC and the Biophysics Branch has focused on the type of instrumentation, safety, power requirements, systems interface, detection methods, sample injection methodology, buffer systems, sample handling, and recording of results for capillary electrophoresis studies. A Model 785A absorbance detector was obtained from Applied Biosystems, Inc. A high sensitivity optical cell assembly supplied by L.C. Packings, San Francisco, CA, includes a CE cell, a fused silica capillary, 75 μm inside diameter and 280 μm outside diameter, coated externally with polyimide, and a reference cell. The analytical length of the capillary is 122 cm, 100 cm from the injection end to the detector cell and 22 cm to the outlet buffer. The Z-cell has a light path of three millimeters. The KSI 1000 Series 100-5 power supply is capable of delivering 100 kV of dc power and is connected to the anode by using standard sparkplug wires and terminals. These terminals on the anode side were mounted into a custom one-quarter inch plexiglass capillary holder especially designed to hold the capillary in place under load. Since power requirements may range up to 40 kV and 90 μA , a plexiglass cover for the entire terminal and connection assembly was designed and custom built for safety shielding at the inlet buffer electrode. The capillary holder, connectors, terminals and safety shielding assemblies were designed by Percy Rhodes and David Donovan and built by Campbell Engineering of Huntsville, AL. Grounding is provided by direct mount to the absorbance detector chassis and by connection directly into the laboratory electrical system ground. A Fluke model 87 multimeter was connected for in-line monitoring of the system. A Kipp and Zonen chart recorder was connected to the detector output for recording results.

Conditioning of the capillary was required before system interfacing and full operation of the unit could be tested. To reduce the amount of direct handling of the capillary and possible breakage, two to three centimeters of a glass capillary was epoxy-welded as a sleeve over both ends. The glass sleeve allowed us to attach a piece of small rubber tubing between an eighteen gauge hypodermic needle on the syringe and the fused silica capillary. The capillary was purged by pumping 1M NaOH for thirty minutes with a syringe pump. The operating buffer of choice, 0.1 M Tris-HCl (pH 8.05, conductivity = $\sim 4800 \mu\text{mhos}$), was then flushed through the capillary. Tests for absorbance detection were made using 20 mM sodium phosphate at pH 2.5. Before the system could be fully operational and brought under load, further cell conditioning was necessary. The cell optics in the detector were burned in by illumination with the deuterium lamp for forty-eight hours at a wavelength of 200 nanometers.

Buffer preparation was carefully controlled by using distilled water and filtration with a 0.22 μm pore size. All fluids were filtered before introduction into the capillary. Sample injection was achieved by using 10 kV of power to enable the sample to flow into the tube on the anode side by electroosmosis. A biotinylated protein molecular weight standard which is prepared for use with gel electrophoresis was used initially for detection. In addition, egg white lysozyme was prepared and run in the CE system. A Hewlett-Packard Model 8452 UV-VIS Spectrophotometer with computer interface was used to provide electropherograms of the buffers and sample prior to the CE runs.

Results and Discussion

We fully expected to experience a "learning curve" with these experiments, but, overall, the results have been somewhat disappointing. Over a period of about three weeks we have experienced several problems relative to the detection equipment and the system as a whole, but particularly with the CE-cell. Some of the problems are listed below.

1. initial filling of the capillary
2. erratic results with absorbance detection
3. current unstable
4. amount of current inconsistent with calculated theoretical values
5. small current spike
6. baseline drift
7. electropherograms show unreliable and inconsistent results
8. possible thermal problems
9. bubbles in the capillary under load
10. sample injection methodology

The first attempt at filling the capillary provided evidence of a problem with the CE-cell. Initial tests of the capillary system under load of 20kV produced a current of between 0.9 μ A to 29.0 μ A. These results were inconsistent with the calculated values of 60-80 μ A while using the high conductivity Tris-HCl. Upon examination of the capillary with a dissecting microscope, an obstruction was observed about ten centimeters from the inlet buffer end. This section of the capillary was sacrificed and the system was tested again under load. Subsequent tests provided excellent results which were consistent with the calculated theoretical values. We were able to run the protein molecular weight standard and record the results on the chart recorder. However, the results of several experiments conducted under the same conditions were unacceptable. Changes in the buffers and sample injection were made. There was some evidence of bubbles in the system. Degassing of all buffers and sample is now standard operating procedure. These precautions eliminated some of the problems we were experiencing, however, the baseline noise or drift continued to be a serious area of concern. Finally, we determined that the CE-cell was defective. At the time of this report the cell has been returned and a new one is being provided by the company.

Recommendations for Further Research

Sample injection may be accomplished in a number of ways, but the recommendation is the one used by Grossman and Soan. A vacuum of 5 in Hg is applied to the cathodic electrode reservoir for two or three seconds while the inlet end of the capillary is immersed in the sample solution (6). On-column detection seems to be the method of choice for high resolution electropherograms (5) (9) (10). Strege and Lagu have also obtained higher resolution electropherograms with the use of coatings of polyacrylamide on the inner surface of the capillary (11). Various buffer systems may be used for better results and cooling of the capillary will eliminate problems which may be thermally induced. Finally, we recommend tests with various samples including the tobacco mosaic virus, lambda DNA, DNA ladders, deproteinated chromosomes of the yeast *S. cerevisiae* and intact chromosomes of *S. pombe*.

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